SUPPLEMENTARY FIGURES NEWELL-LITWA et al.:

S. Figure 1: Characterization of VAMP7-TI Monoclonal Antibody.

A) Bacterially purified GST, VAMP2-GST, and VAMP7-TI-GST constructs are all recognized by a GST antibody. However, monoclonal antibodies against either VAMP2 or VAMP7-TI specifically recognize their substrates (lanes 2 and 3 respectively). B) VAMP7-TI siRNA-treatment of HELA specifically reduces VAMP7-TI protein levels, as detected by loss of VAMP7-TI immunoreactivity (lane3). Transferrin receptor (TfrR) and β-actin are unaffected by VAMP7-TI siRNA treatment. C) VAMP7-TI siRNA reduces VAMP7-TI signal detection in fixed HELA cells. Scale Bar = 5μm.

S. Figure 2: Synaptic Vesicle SNARE, VAMP2, Co-localizes with AP-3-Sorted Synaptic Vesicle and Lysosomal Proteins.

Fixed PC12 cells co-immunostained for synaptic vesicle SNARE, VAMP2 with one of the following: AP-3δ subunit, lysosomal proteins (VAMP7-TI, PI4KIIα, and CD63), and synaptic vesicle protein, ZnT3. Figure 1B reports quantification of co-localization between VAMP2 and ZnT3, PI4KIIα, and CD63. Note that the cell stained with ZnT3 and VAMP2 was triple labeled with anti VAMP7-TI antibodies. The same cell with the ZnT3 and VAMP7-TI channels is shown in Figure 1. Scale Bar = 5μm.

S. Figure 3: Syntaxin 8 Colocalization with Synaptic Vesicle and Lysosomal SNAREs.

Fixed PC12 cells co-immunostained for the lysosomal SNARE, syntaxin 8, and one of the following markers: the lysosomal SNARE vti1b; the cognate lysosomal SNARE, VAMP7-TI; and the synaptic vesicle SNARE, VAMP2. Figure 1B reports quantification of co-localization values. Scale Bar = 5μm.

S. Figure 4: Negligible Co-Localization of VAMP7-TI and VAMP2 with Golgi and Late Endosomal Markers.

Fixed PC12 cells co-immunostained for A) VAMP7-TI and cis Golgi marker, GM130, or trans Golgi marker, TGN38; B) VAMP2 and GM130 or TGN38; C) late endosomal marker, LBPA, and VAMP2 or VAMP7-TI. Quantification of these immunofluorescence pairs (Figure 1B) reveals minimal co-localization. Scale Bar = 5μm.

S. Figure 5: Quantification of Synaptic Vesicle and Lysosomal AP-3 Cargoes Co-Localization in Mouse Primary Neurons.

Data were obtained from images such as those presented in Figure 4. Data are depicted as probability plots in which the extent of colocalization between two markers is linearly represented in the y axis and the % of the population, arranged from the lowest to the highest colocalization values, are depicted logarithmically in the x axis. Fifty percent in the X axis establishes the median of the population (indicated by the gray arrow). For example, the median of colocalization between VAMP2 and synaptophysin is 74.5%. (Cell Body, left panel, red triangles, V2/Sphysin). Thus, half of all the VAMP2 and synaptophysin co-localization data fall below the median and they range between 30 to 74.5% colocalization. We defined the upper limits of colocalization by the signal overlap between two synaptic vesicle proteins, synaptophysin and VAMP2. Each data point represents the quantified co-localization of one z-plane. Three z planes represent each cell. Co-localization for neuronal cell bodies (left and middle panels): VAMP2/Synaptophysin (V2/Sphysin n=14), VAMP2/VAMP7-TI (V2/V7 n=76), VAMP2/LAMP1 (V2/LAMP1 n=45), VAMP2/LBPA (V2/LBPA n=10), VAMP2/AP-3 delta (V2/AP-3 δ n=17), VAMP7-TI/LAMP1 (V7/LAMP1 n=44), VAMP7-TI/LBPA (V7/LBPA n=10), VAMP7-TI/AP-3 delta (V7/AP-3 δ n=20). Co-localization for all processes (right panel): V2/V7 (n=78), V2/Sphysin (n=15) and V2/LBPA (n=10). Dendrite and axons are analyzed from the following number of cells: V2/V7 n=5 and n=5, respectively. Dendrites were identified by MAP2 staining whereas axons were defined as MAP-2-negative processes. Asterisks represent $p<0.0001$ Kolmogorov-Smirnov test.

Within the neuronal cell body, lysosomal AP-3 cargo co-localization (VAMP7-TI/LAMP1) and synaptic vesicle/lysosomal co-localization (VAMP2/VAMP7-TI and VAMP2/LAMP1) exceeds the co-localization observed with late endosomal compartments, as indicated by LBPA. VAMP2/Synaptophysin co-localization indicates the upper limit of co-localization. Additionally, both VAMP2 and VAMP7 significantly co-localize with AP-3 in cell bodies.

S. Figure 6: Synaptic Vesicle Fractions from Mouse Brains do not Contain Late and Recycling Endosome Markers.

S2 fractions from C57B or Muted+/mu were resolved in 5-25% glycerol gradients. Gradient fractions were resolved by SDS-PAGE and probed with antibodies against

synaptophysin (Sphysin), syntaxin 13 (Synt13, recycling endosome), syntaxin 8 (Synt8, late endosome), Vti1b (late endosome). Sialin is a well characterized lysosomal protein (Sagne and Gasnier, 2008). Lanes 1 and 2 contain P1 and P2 fractions for a positive signal.

S. Figure 7: Glycerol Gradient Velocity Sedimentation of Synaptic Vesicle Fractions from AP-3-deficient Mouse Brains.

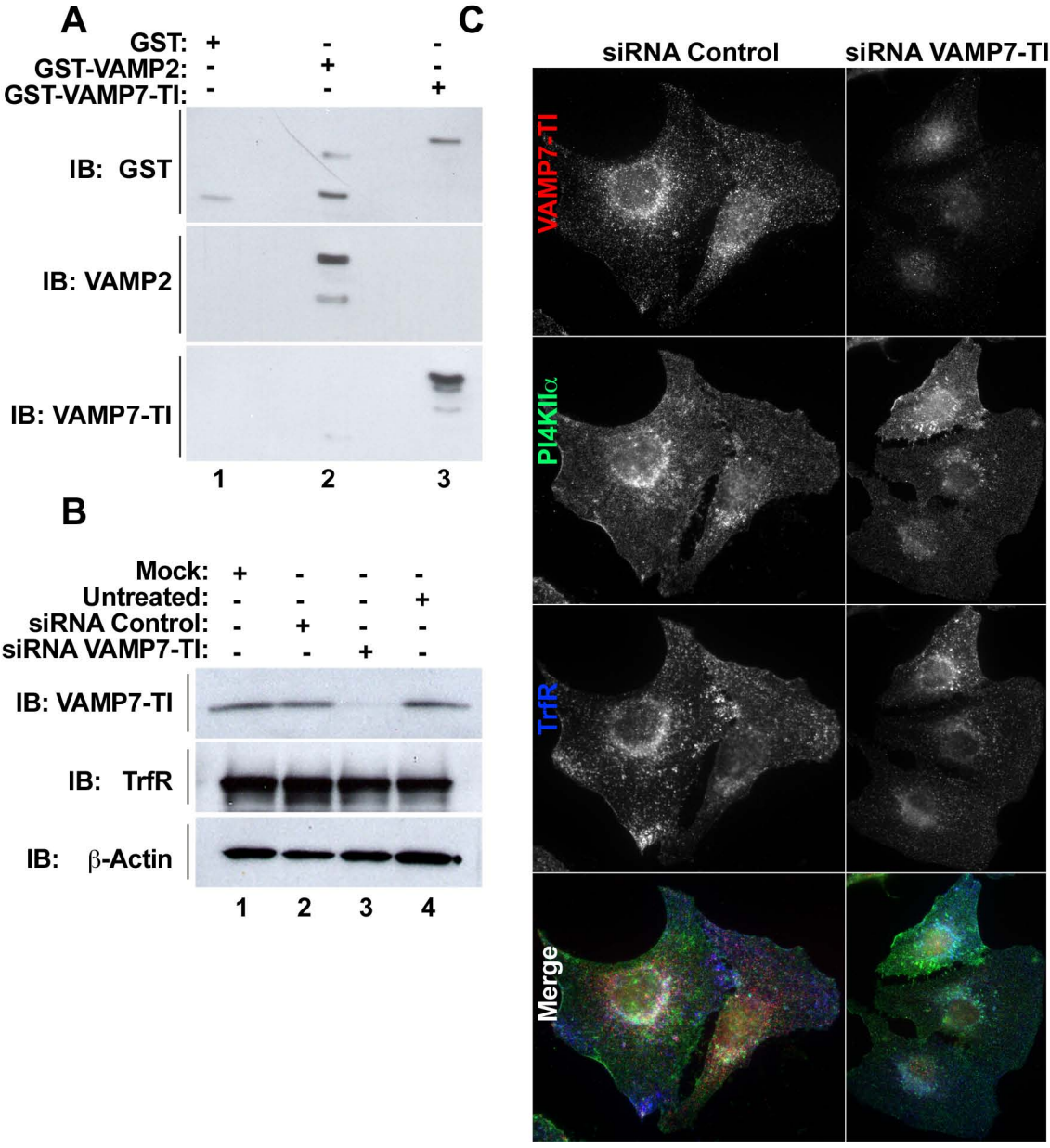
Representative western blots of 5-25% glycerol gradients of S2 fractions from wild type C57B, *Ap3b1*$^{-/-}$, and *Ap3b2*$^{-/-}$ mouse brain. Synaptophysin indicates that synaptic vesicle fractions peak in the middle of the gradient, and also serves as a loading control for the different mice. Figure 7 reports quantification of the glycerol gradient fractions. Glycerol gradient density is indicated at the bottom of the figure, with larger vesicle fractions migrating toward 25% glycerol.

S. Figure 8: Glycerol Gradient Velocity Sedimentation of Synaptic Vesicle Fractions from BLOC-1 Deficient (*muted*) Mouse Brains.

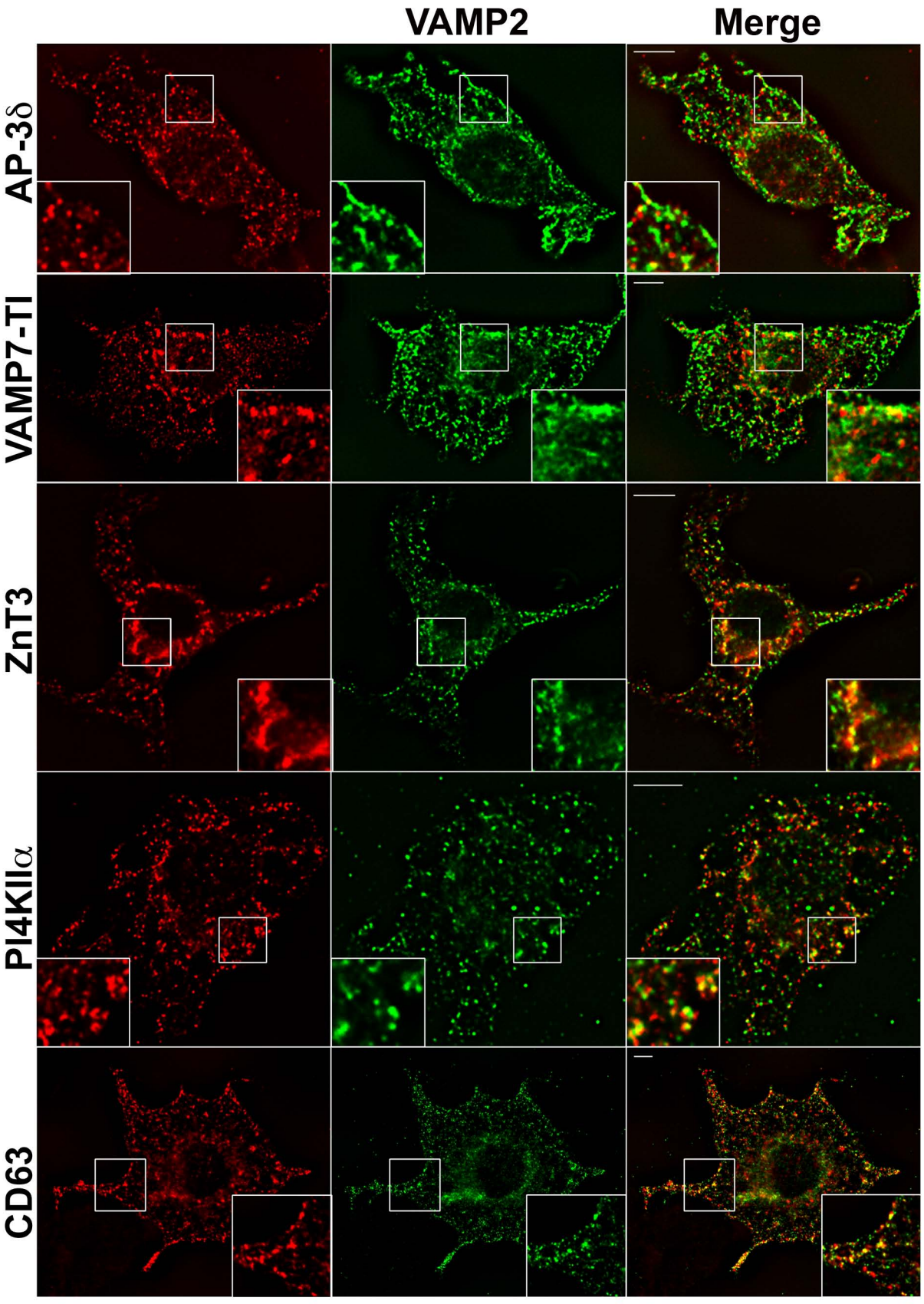
Representative western blots of 5-25% glycerol gradients of S2 fractions from heterozygous control, *Muted*$^{+/mu}$, and *muted* (*Muted*$^{mu/mu}$) mouse brains. Synaptophysin indicates that synaptic vesicle fractions peak in the middle of the gradient, and also serves as a loading control for the different mice. Figure 8 reports quantification of the glycerol gradient fractions. Glycerol gradient density is indicated at the bottom of the figure, with larger vesicle fractions migrating toward 25% glycerol and identified toward the left of the western blot, and cytoplasmic fractions migrating toward 5% and identified toward the right of the western blot.

S. Figure 9: AP-3 and BLOC-1 Deficiency Do Not Globally Affect Synaptic Vesicle and AP-3 Sorted Lysosomal Proteins in Mouse Brain.

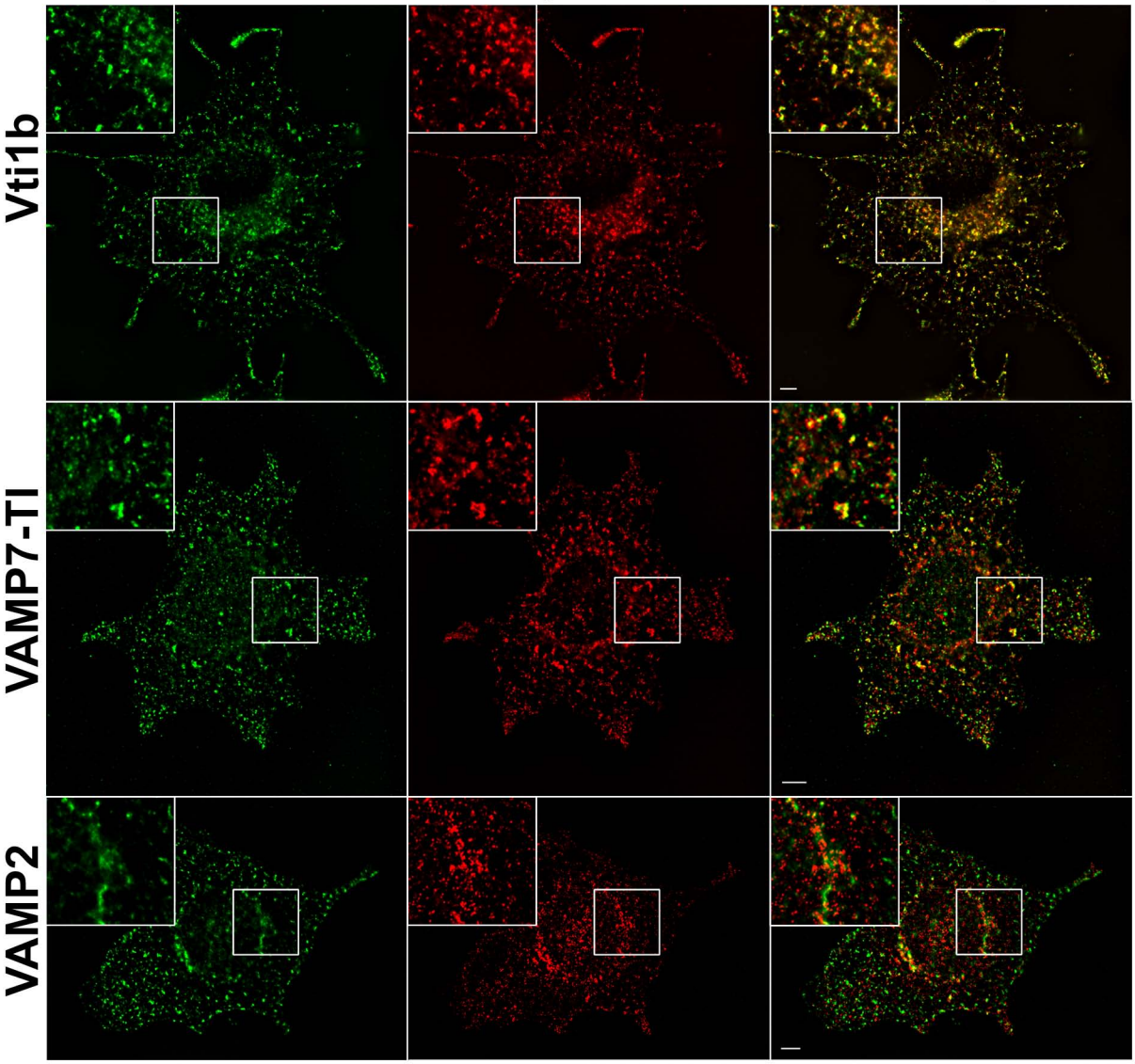
Western blots of pellets (P1, P2) and final supernatant (S2) obtained from differential centrifugation of wild type C57B, *Ap3b1*$^{-/-}$, and *Ap3b2*$^{-/-}$ mouse brain as well as *Muted*$^{+/mu}$, and *Muted*$^{mu/mu}$ brains. Western blots were probed for the AP-3-sorted lysosomal and synaptic vesicle proteins. AP-3 σ3 indicates that AP-3 levels are most dramatically reduced in *Ap3b2*$^{-/-}$. α-tubulin serves as a loading control.


A
GST: + - -
GST-VAMP2: - + -
GST-VAMP7-TI: - - +
IB: GST
IB: VAMP2
IB: VAMP7-TI
1 2 3
B
Mock: + - - -
Untreated: - - - +
siRNA Control: - + - -
siRNA VAMP7-TI: - - + -
IB: VAMP7-TI
IB: TrfR
IB: β-Actin
1 2 3 4
C
siRNA Control
siRNA VAMP7-TI
VAMP7-TI
PI4KIIα
TrfR
Merge

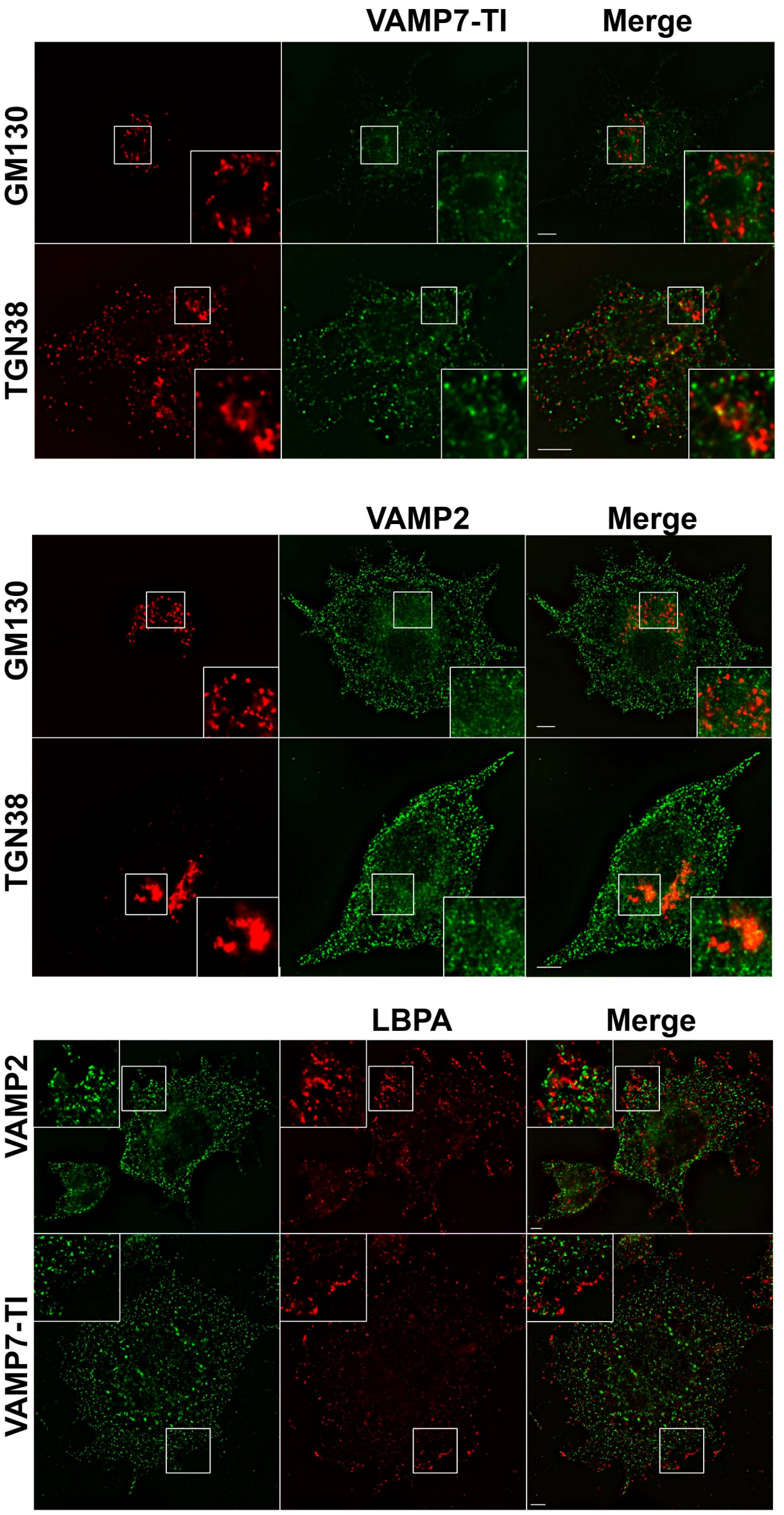
SFig. 1



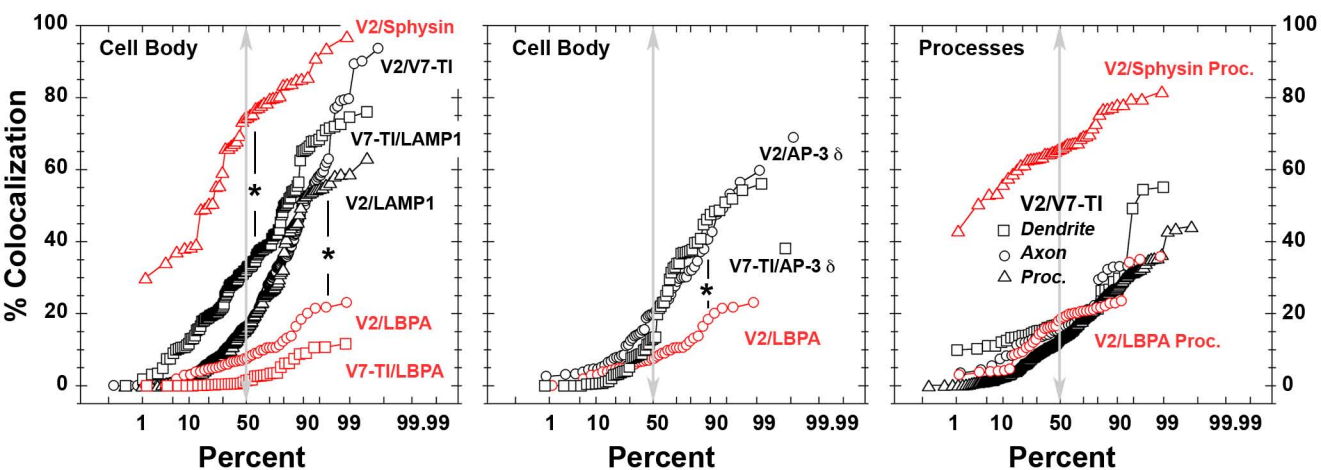
SFig. 2



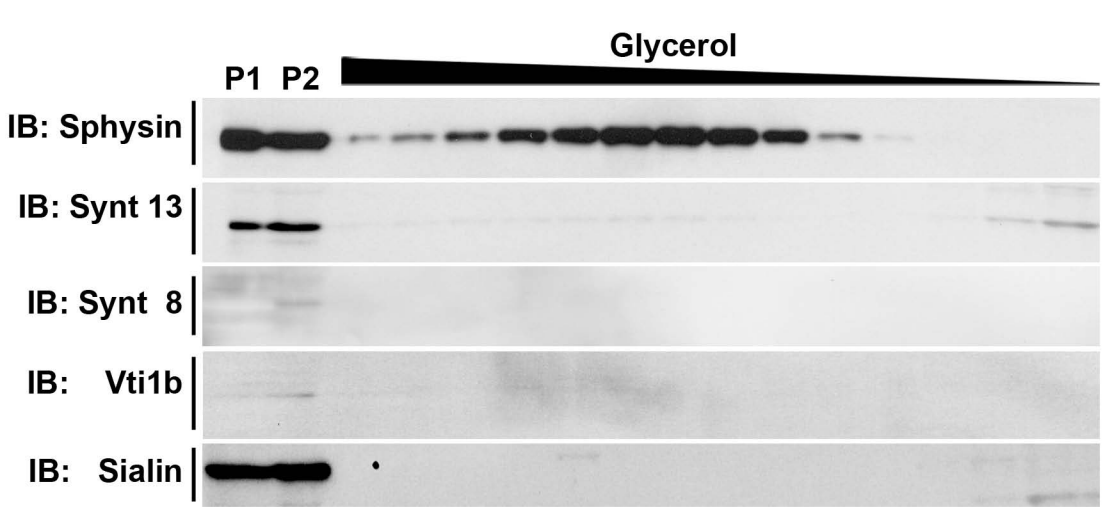
SFig. 3


VAMP7-TI
Merge
GM130
TGN38
VAMP2
Merge
GM130
TGN38
LBPA
Merge
VAMP2
VAMP7-TI

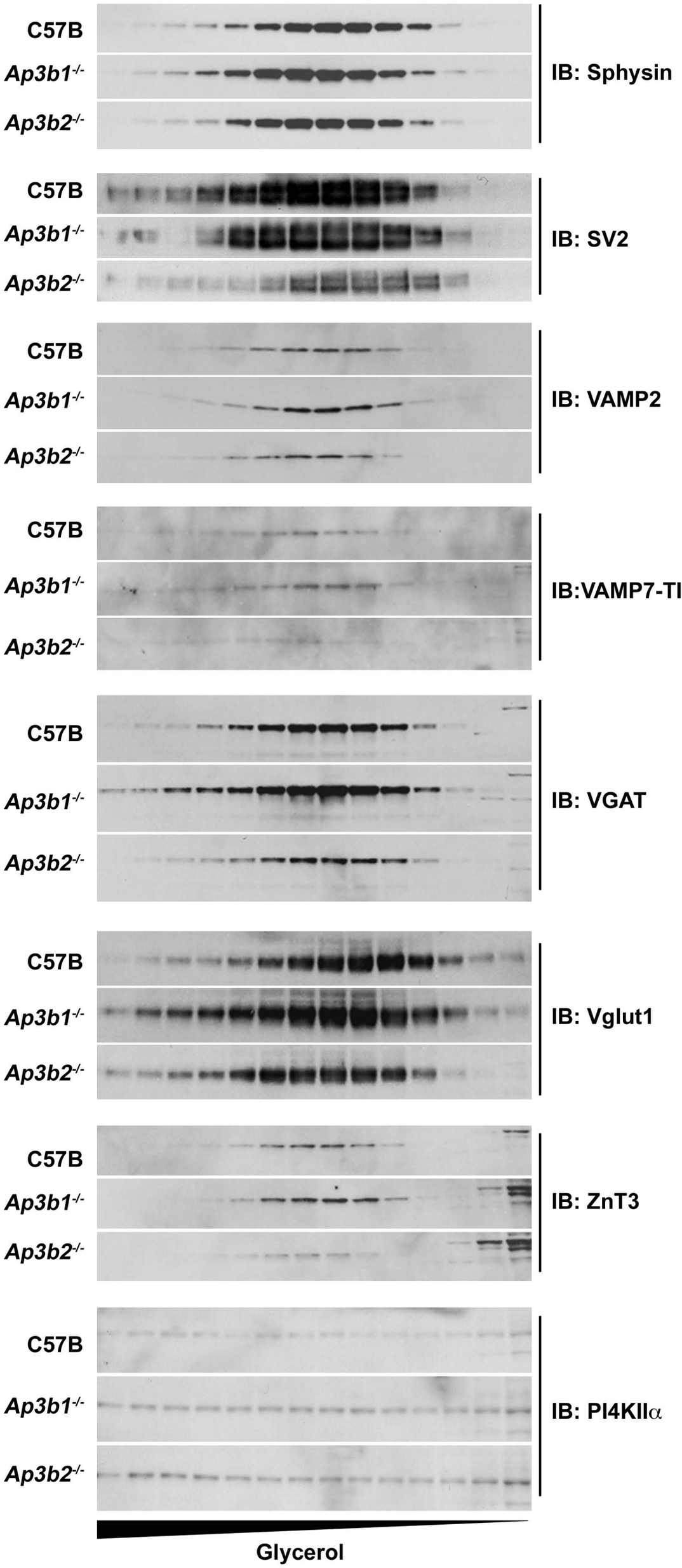
SFig. 4


% Colocalization
Cell Body
V2/Sphysin
V2/V7-TI
V7-TI/LAMP1
V2/LAMP1
V2/LBPA
V7-TI/LBPA
Percent
Cell Body
V2/AP-3 δ
V7-TI/AP-3 δ
V2/LBPA
Percent
Processes
V2/Sphysin Proc.
V2/V7-TI
Dendrite
Axon
Proc.
V2/LBPA Proc.
Percent
0
20
40
60
80
100
1
10
50
90
99
99.99

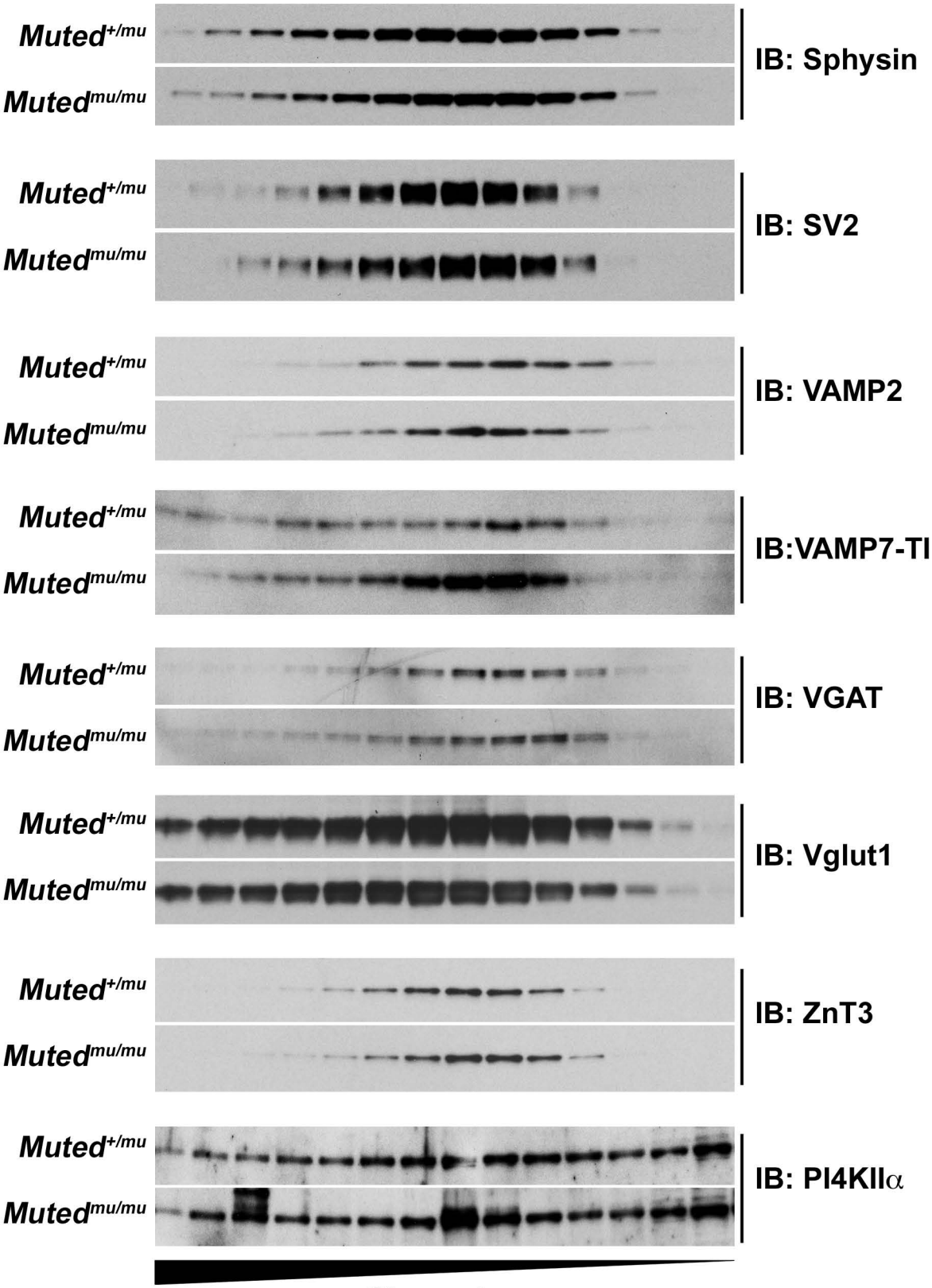
SFig. 5


Glycerol
P1 P2
IB: Sphysin
IB: Synt 13
IB: Synt 8
IB: Vti1b
IB: Sialin

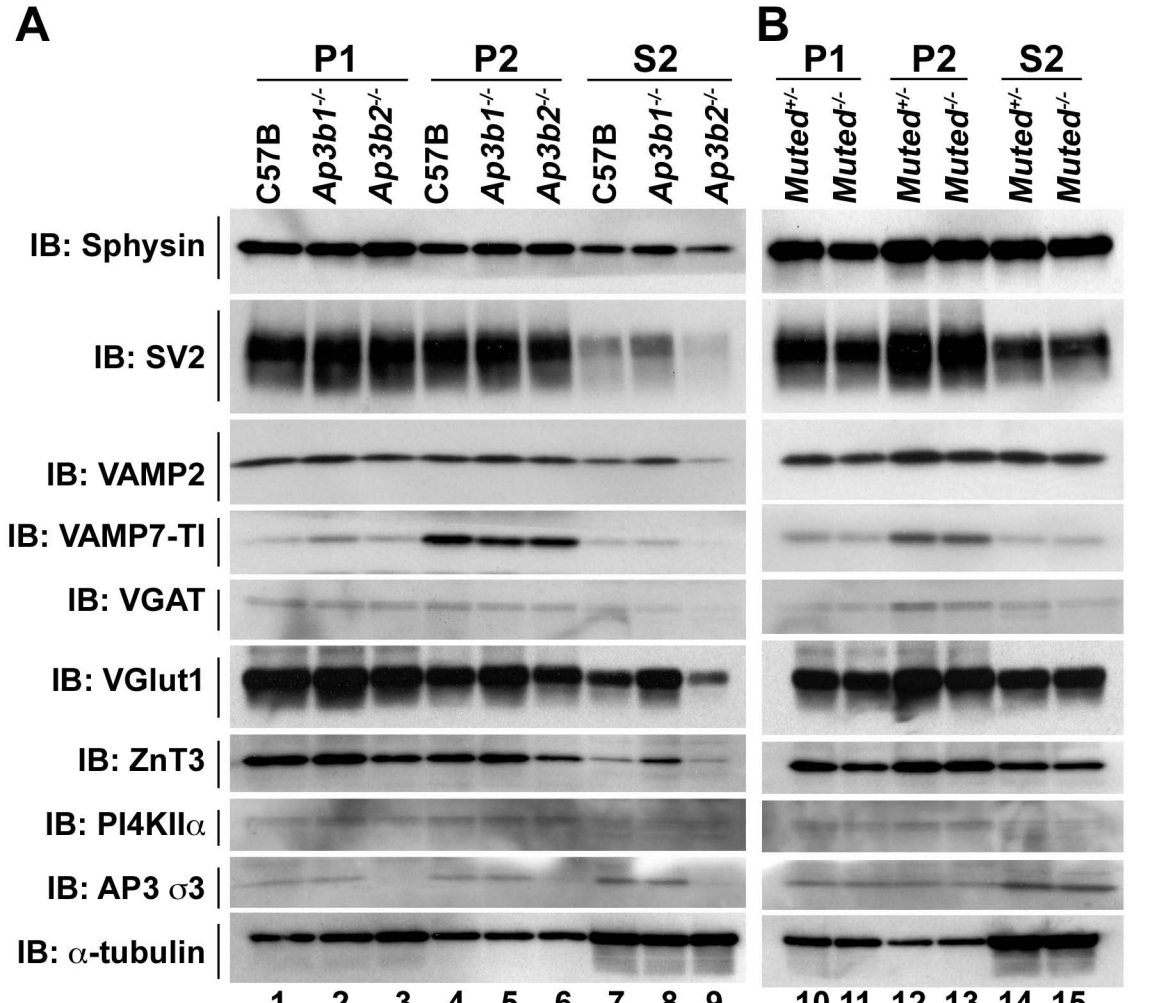
SFig. 6


C57B
Ap3b1-/-
Ap3b2-/-
IB: Sphysin
C57B
Ap3b1-/-
Ap3b2-/-
IB: SV2
C57B
Ap3b1-/-
Ap3b2-/-
IB: VAMP2
C57B
Ap3b1-/-
Ap3b2-/-
IB:VAMP7-TI
C57B
Ap3b1-/-
Ap3b2-/-
IB: VGAT
C57B
Ap3b1-/-
Ap3b2-/-
IB: Vglut1
C57B
Ap3b1-/-
Ap3b2-/-
IB: ZnT3
C57B
Ap3b1-/-
Ap3b2-/-
IB: PI4KIIα
Glycerol

SFig. 7


Muted+/mu
Mutedmu/mu
IB: Sphysin
Muted+/mu
Mutedmu/mu
IB: SV2
Muted+/mu
Mutedmu/mu
IB: VAMP2
Muted+/mu
Mutedmu/mu
IB:VAMP7-TI
Muted+/mu
Mutedmu/mu
IB: VGAT
Muted+/mu
Mutedmu/mu
IB: Vglut1
Muted+/mu
Mutedmu/mu
IB: ZnT3
Muted+/mu
Mutedmu/mu
IB: PI4KIIα
Glycerol

SFig. 8


A
B
P1
P2
S2
C57B
Ap3b1-/-
Ap3b2-/-
Muted+/-
Muted-/-
IB: Sphysin
IB: SV2
IB: VAMP2
IB: VAMP7-TI
IB: VGAT
IB: VGlut1
IB: ZnT3
IB: PI4KIIα
IB: AP3 σ3
IB: α-tubulin
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

SFig. 9